Mouse helpers ensure maternal-infant survival

Luisa Schuster[1-4], Renee Henderson[1-3], Violet J. Ivan[1-4], Asha Caslin, Gurket Kaur, Shreya Sankar[1-3], Deepasri Ananth[1-3], Matilda Kirk[1-3], Latika Khatri[1-4], Paola Leone[1-3], Karen E. Adolph[4,5], Robert C. Froemke[1-4]*, and Adam Mar[1,2]*

[1] Neuroscience Institute,
[2] Department of Neuroscience and Physiology,
[3] Department of Otolaryngology, New York University Grossman School of Medicine, New York, NY, USA.
[4] Center for Neural Science,
[5] Department of Psychology, New York University, New York, NY, USA.
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* Correspondence to: R.C. Froemke, phone: 212-263-4082; email: robert.froemke@med.nyu.edu and: A. Mar, phone: 212-263-9295; email: adam.mar@nyulangone.org

Parental care is required for offspring health and survival, as infants are largely helpless and require near-continual oversight for extensive periods. Parents must balance caretaking behaviors with other activities for their own survival such as thermoregulation and foraging, necessarily involving consequential decision-making and periods of pup neglect. We built a new system for long-term continuous recordings of mouse homecage behaviors, monitoring single wild-type or oxytocin receptor knockout (OXTR-KO) dams and offspring over four litters. Here we show that experienced female mouse mothers use behavioral mechanisms to ensure maternal and infant survival of other consociating animals. Many wild-type mothers had high litter survival rates ('high-pup-survival' dams), but about a third of wild-types had little to no pups survive ('low-pup-survival' dams), and this did not change across litters. Low-pup-survival dams generally avoided pups and nests. However, if low-pup-survival dams were temporarily co-housed with a high-pup-survival dam and her litter, their litter survival rates then enduringly increased due to enhanced attention toward pups and nests. In contrast, almost all singly-housed OXTR-KOs died in childbirth and their pups perished after prolonged parturition. Co-housing with another female prevented maternal and infant mortality, with the other female removing and cleaning pups from the pregnant dam. These results show that even under well-controlled housing conditions, mouse maternal care can be remarkably variable, with continued nest rebuilding as a major factor for post-parturition litter survival. While some single mothers continually lose their litters or die in labor, maternal and infant survival can improve through interactions with other experienced females.

Main Text

Parental behavior is essential for the well-being of offspring and the survival of the species (Dulac et al., 2014; Rilling and Young, 2014). Adult animals can become highly sensitive to the needs of their infants to adequately care for them, as infants require intense continuous protection for days to years depending on species. Mouse pups in particular rely on their mother for sustenance, thermoregulation, and protection, supported by successful nest-building (Leon et al., 1978; Lynch and Possidente, 1978; Gaskill et al., 2012). However, pup mortality can result if dams are neglectful or provide inadequate care during the postnatal period. Parental animals must balance caregiving behaviors with other behaviors required for their own survival, making single parenting especially challenging. Previous studies of rodents and other species report a remarkably high degree of infant mortality, both outdoors in native habitats and animals housed in laboratory conditions- although it remains controversial how much infant mortality occurs for isolate vs communally-rearing mothers (König, 1993; Manning et al., 1995; Ferrari et al., 2019; Capas-Peneda et al., 2020; Brajon et al., 2021). We aimed to resolve when and how pup mortality occurs, if it is due to parental neglect or other factors, and if mouse mothers might learn from certain experiences to improve maternal care over subsequent litters. We also examined oxytocin receptor knockout mouse mothers compared to wild-types, to clarify how oxytocin receptor signaling might be important for maternal-infant health.

We designed a high-resolution system to monitor parental care and family life in mice with multiple cameras, recording from homecages continuously 24 hours/day for weeks to months across consecutive litters (**Fig. 1A**). Individual cages were contained within custom-designed environmental control boxes, containing at least two camera mounts to record from the top, side,

63 and underneath the nest. We also used infrared cameras to record in darkness and a thermal
64 imaging camera to monitor temperature. Video was combined with an inexpensive, open-source
65 system to control LED light-strips to provide a naturalistic light/dark cycle, together with
66 temperature and humidity sensors connected to an Arduino circuit board that controls two fans,
67 and adjusts their speed to maintain the enclosure temperature at 26-28 °C (**Fig. 1B**), a level close
68 to adult mouse thermoneutrality of 30°C (Król & Speakman, 2003; Gaskill et al., 2012). Data were
69 saved to local servers and streamed remotely for machine learning-based quantitative behavioral
70 analysis along with annotations by several independent experienced human raters.
71
72 We monitored a total of 64 single-housed wild-type C56Bl/6 female mice continuously over 3-4
73 months, from first mating and first litter through subsequent breeding and litters (**Fig. 1C**, top).
74 Although many different maternal behaviors were documented and scored over the postnatal
75 period, we focused on interactions with pups and the nest in the first few days of each litter (**Fig.**
76 **1C**, bottom). This was due largely to surprisingly high pup mortality even in the protected
77 conditions of the lab vivarium and standardized housing enclosures we developed. We quantified
78 litter survival from the moment of birth over the first 24 postnatal hours, in which most mortality
79 was observed. For the first cohort of 21 mice, the average survival of the first litter was 60.5±9.7%.
80 However, litter survival was clearly bimodal—9/21 first-time dams had 100% pup survival, and
81 6/21 dams had 0% pup survival (**Fig. 1D**, Litter 1). We refer to dams with ≥50% litter survival as
82 'high-pup-survival' dams, and dams with <50% pups surviving as 'low-pup-survival' dams.
83
84 We hypothesized that for low-pup-survival dams, litter survival rates might improve over
85 subsequent litters as dams learned from experience or had other forms of maternal adaptation in

86 the service of improved caregiving. However, this was not the case. As shown in **Figure 1D**, litter
87 survival of singly-housed wild-type C57Bl/6 mice continued to be bimodal, with 13/21 high-pup-
88 survival dams and 8/21 low-pup-survival dams for litter 2, 8/21 low-pup-survival dams for litter
89 3, and 6/12 low-pup-survival dams for litter 4 (**Fig. 1D**, left). With the exception of a single animal,
90 high-pup-survival dams continued to have high litter survival rates across all 3-4 monitored litters,
91 and low-pup-survival dams consistently lost most or all of their litters across all monitored litters
92 (**Fig. 1D**, right). This meant that the litter survival rate of the first litter predicted with high
93 accuracy the survival of subsequent litters (**Fig. 1E**; N=21, $r \geq 0.8$, $p<0.001$).
94
95 Inspection of video recordings showed that almost none of the litter mortality observed in either
96 high- or low-pup-survival dams was due to aggression or mauling of otherwise healthy pups
97 (observed in 5/64 dams). Instead, low-pup-survival dams neglected pups and nests after initial
98 nest-building was complete. In contrast, high-pup-survival dams consistently re-engaged in nest-
99 building, especially because entering and leaving the nest sometimes disrupted nest integrity. We
100 found that hand-scoring nest quality 1-3 days prior to parturition also predicted litter survival
101 across dams (**Fig. 2A**).
102
103 The importance of nest quality likely reflects the function of nests to keep pups insulated especially
104 when the dam is not present in the nest, because pups do not reliably thermoregulate their body
105 temperature and can perish from hypothermia (Weber and Olsson, 2008). However, it proved
106 challenging to reliably hand-score nests over extended periods, because nest structure was dynamic
107 and the visual features of a nest might not perfectly relate to the thermal insulative qualities that
108 nests must provide. Therefore, we used thermal imaging to monitor pup temperature directly. This

required us to segment the nest from the video recording and use it to isolate the temperature of the pups within the nest (**Fig. 2B**).

We monitored pup temperature during periods when dams made excursions from the nest. This happened frequently as mother animals left to forage for food and water, and to regulate their own body temperature as they can overheat if they stay too long on the nest (Leon et al., 1978; Gamo et al., 2016; Król and Speakman, 2019). We focused on moderate-duration excursions of >23 seconds, averaging pup temperature and heat loss over this period. We found that both the initial temperature and the temperature drop predicted pup mortality: higher tonic temperatures and less average heat loss were correlated with higher pup survival (**Fig. 2C,D**).

On average the total time spent on the nest was unrelated to litter survival rates (**Fig. 2E**). Initial time spent nest-building also was uncorrelated with litter survival (**Fig. 2F**). Instead, the main post-partum predictor of pup survival was amount of time the dam spent rebuilding the nest (**Fig. 2G**). Low-pup-survival dams typically spent some time nest-building initially, but then gave up, and subsequently lost most or all of their offspring within hours. High-pup-survival dams continued to adjust and rebuild the nest especially after leaving the nest and disrupting nest integrity. We also noticed that many high-pup-survival dams stocked food pellets near the nest (**Fig. 2H**). This seemed less related to shorter foraging times and more related to structural bolstering of nest wall integrity due to the weight of the food pellets.

It was surprising that over multiple rounds of breeding, low-pup-survival dams did not increase attention to pups and time spent rebuilding their nests. We wondered if low-pup-survival dams

132 somehow could not provide adequate caregiving under any circumstance, or if instead perhaps
133 with different experiences these animals could become less neglectful and have higher survival
134 rates. Previously we documented the emergence of alloparenting in initially-neglectful and pup
135 averse virgin female C57Bl/6 mice, after being co-housed with experienced dams and litters
136 (Marlin et al. 2015; Carcea et al. 2021). In this case for alloparenting, behavioral changes in the
137 virgin were due to encouragement or coercion by the experienced dam, who shepherded or forced
138 the virgin to spend time in the nest until eventually the virgin became a successful alloparent.
139
140 We reasoned that similar parental behavior might emerge if we co-housed an initially low-pup-
141 survival dam with an experienced dam and her litter. We identified a separate cohort of low-pup-
142 survival dams with 0% litter survival at litter 1 and <50% litter survival for litter 2. Prior to mating
143 these animals for litter 3, we co-housed some of these animals for 16-20 days with an experienced
144 high-pup-survival dam and litter; other animals were kept isolated for a similar duration. We then
145 bred these animals and monitored pup survival for litters 3 and 4 when they were returned to being
146 singly-housed in our behavioral monitoring chambers (**Fig. 3A**).
147
148 We found that temporarily co-housing 'low-pup-survival' dams with another experienced mother
149 led to much higher survival rates of litters 3 and 4, compared to the other animals who were just
150 kept isolated for 16-20 days (**Fig. 3B**; red, co-housed dams, litter 3 survival: 74.2±6.6%, N=17;
151 black, isolate dams, litter 3 survival: 15.4±7.3%, N=9, $p<10^{-5}$ compared to litter 3 survival of co-
152 housed animals, Student's unpaired two-tailed t-test). Inspection of video recorded during the co-
153 housing period revealed that while the low-pup-survival dams were initially pup-averse, within
154 hours to days they began interacting with pups and helped with childcare. This seemed to be due

155 to behaviors of the other experienced dam, who shepherded the low-pup-survival dam into the nest
156 and seemingly demonstrated aspects of maternal care (Carcea et al. 2021). This improvement of
157 maternal care in the low-pup-survival dams did not seem to be due to, e.g., social buffering effects
158 from co-housing with any other animal. Co-housing the low-pup-survival dam between litters 2
159 and 3 with either a pup-naïve virgin female (N=8) or an experienced paternal male (N=9) did not
160 improve pup survival for litter 3 (**Fig. 3C**). Co-housing in these cases occurred without pups, as
161 none of the adult animals would be lactating.
162
163 Finally, we wondered if our system would be generally useful for thorough phenotyping of
164 maternal care, infant survival, and other details of mouse family life across different genotypes or
165 other variables of interest. As a test case, we studied oxytocin receptor knockout mice, as there is
166 continued interest and some controversy in the range of behavioral and physiological phenotypes
167 of animals lacking oxytocin receptors (Berendzen et al., 2023). As our specific question of interest,
168 we wondered how animals reported to not have uterine contractions could have normal parturition
169 (Takayanagi et al., 2005).
170
171 The first cohort of receptor knockout animals we examined were singly-housed and nulliparous
172 knockout females placed in our housing system after the first round of mating and breeding.
173 Animals turn in circles during labor, clearing the cage floor of bedding and facilitating
174 unobstructed video recording from underneath the homecage during this time. To our surprise,
175 singly-housed oxytocin receptor knockout animals struggled for hours to days in labor, with pups
176 becoming stuck in the birth canal and dams attempting to mechanically push pups out or pull them
177 out with their mouth or paws. The prolonged and difficult labor prevented them from being able

178 to clean pups of the amniotic sac. As a consequence, all pups born to singly-housed oxytocin
179 receptor knockout dams died (**Fig. 4A,B**, 'Non', N=7, 0% pup survival for all litters). Likely
180 because of this protracted labor, almost all singly-housed receptor knockout dams died as well
181 (**Fig. 4C,** 'Non', 6/7 oxytocin receptor dams expired).
182
183 Much mouse breeding occurs in group-housed cages (e.g., for 'harem breeding', as well as to
184 reduce number of animal cages and cage costs). Additionally, as oxytocin receptor knockout mice
185 do not have milk ejection during nursing (Takayanagi et al., 2005), for pups to survive post-
186 parturition animals must be co-housed with a lactating female. Thus the other cohort of oxytocin
187 receptor knockout females we examined were co-housed with a pregnant wild-type female. The
188 oxytocin receptor knockout dams also had prolonged and difficult labor, but when the first pup
189 breached and got stuck in the birth canal, we observed remarkable changes in behavior of the co-
190 housed pregnant wild-type mouse. The other female approached the dam in labor, and seemed to
191 act as a 'midwife', helping to remove stuck pups with her mouth and paws, and cleaning pups of
192 the amniotic sac so they could begin to breathe (**Fig. 4D,E**). These helping behaviors seemed vital
193 to maternal-infant survival. In all cases, pup mortality was drastically reduced and litter survival
194 rates were quite high (**Fig. 4A**; '+Dam', 90.2±3.5% pup survival per litter, N=10 litters, $p<10^{-5}$
195 compared to non-co-housed receptor knockout litters, Student's two-tailed unpaired t-test). 9/10
196 co-housed oxytocin receptor knockout dams also survived labor, compared to 1/7 singly-housed
197 dams (**Fig. 4C**, '+Dam', $p<0.003$, Fisher's two-tailed exact test).
198
199 Our study provides insights into mouse maternal behavior over time and across consecutive litters,
200 the impact of the nest and its insulating capacity on pup survival, and the importance of interactions

201 and behaviors of other experienced mouse mothers particularly with animals who would otherwise
202 lose their pups and die in childbirth. These observations resulted from the 'documentary' approach
203 to monitoring animal behavior continuously over extended periods of days to months, with
204 multiple cameras including under the nest. This was essential to accurately capture moments of
205 parturition, to identify the number of pups delivered by each dam, the caregiving behaviors or
206 neglect by the mother, and behaviors between dyads of animals in the service of pup survival. Our
207 system was designed to be low-cost and reduce the amount of intervention or maintenance required
208 to monitor homecage behavior, and thus is similar in these regards to other behavioral systems
209 starting to be developed by other groups (Benedict and Cudmore, 2023; Zhang et al., 2023).
210
211 Our findings indicate that even under the controlled and protected conditions of a laboratory
212 vivarium, childcare can be immensely challenging and taxing, requiring continued effort on the
213 part of caregivers to ensure pups are fed and warm. Under the conditions of our environmentally-
214 controlled home cages, approximately a third to a half of C57Bl/6 mouse mothers were observed
215 to lose most to all of their litters due to neglect of pups and nest. The nest structure was quite
216 dynamic, needing to be repeatedly adjusted or rebuilt due to the movement of the mother in and
217 out of the nest, and the motion of the pups. Thermal imaging was important to relate this aspect of
218 parental behavior to litter survival, as pups rapidly dropped in temperature without a well-
219 constructed insulating nest and died within minutes.
220
221 One potential concern is that the extent of pup mortality and relatively high prevalence of low-
222 pup-survival dams is a consequence of sustained single-adult housing especially combined with
223 the stress of childcare. A related caveat is that our studies were performed exclusively in C57Bl/6

mice, and it is known that there are differences in parental behaviors across rodent strains (Ward, 1980). Even accounting for the genetic background, within the C57Bl/6 strain there was considerable bi-modality in childcare abilities, which might reflect other individual differences in gene expression and/or neural responses to infant distress cues that will need to be further documented. Most wild species of mice are believed to live in multi-female colonies (Ferrari et al., 2019) where perhaps many different adults can participate in childcare, tending to pups and nests, and shepherd or help other inexperienced adults take care of offspring. However, little is known about the fine-scale adult social dynamics of colony organization and how this relates to communal child-care. The system we have developed enables a systematic and parametric exploration of different variables related to social structure and housing conditions, and promises to reveal much more about the family life of lab mice and other species.

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309 **Materials and Methods**

310 All procedures were approved under NYU Grossman School of Medicine IACUC protocols.

311

312 **System for Long-Term 24/7 Behavioral Analysis.** For long-term monitoring of the acquisition
313 and expression of maternal behavior in single-housed animals, we used 8–10-weeks-old C57BL/6
314 naïve virgin female mice. No statistical methods were used to predetermine sample size. The
315 experiments were not randomized. The behavioral raters were blinded to litter survival rate,
316 allocation during co-housing, and outcome assessment.

317 A virgin female mouse was housed alone in a 27.94 × 27.94 × 27.94 cm plexiglass
318 homecage covered with plentiful bedding material, food pellets, hydrogel and/or a water bottle
319 (refreshed every 3-5 days), and one Nestlet. We began by recording continuous video of the naïve
320 virgin behavior while housed alone for up to 5 days, before introducing an experienced C57BL/6
321 male mouse for breeding afterwards. The male mouse was removed from the enclosure after
322 successful intercourse, confirmed through visual examination of video footage, typically after 24-
323 48 hours. For further confirmation of mating, when removing the male mouse from the enclosure,
324 we also checked the virgin for a vaginal plug and we closely monitored the female mouse for
325 physical and behavioral changes typical of a gravid animal. If by day 10 post-mating, the female
326 mouse did not show weight-gain or weight-redistribution, a new experienced male was introduced
327 in the homecage for mating.

328 Dams were allowed to stay with the pups until weaning on P21 in the same homecage.
329 After that, the female was placed back in the enclosure for a few days and mated again, following
330 the same procedure as with the first litter. This was performed for four consecutive litters.

331

Video Acquisition. Video recordings were acquired from 1-3 cameras (OV5647 sensor) sensitive to infrared light and outfitted with 160 FOV wide-angle lenses and placed either above the enclosure (overhead view), perpendicular to the nest (nest view), or below the nest (bottom-up view). Each camera was connected to a Raspberry Pi 4 single board computer that captured, streamed, and saved uninterrupted video from the enclosures (30 frames per second) to a local server using the Python library OpenCV. For some animals, videography was performed continuously at all times up to weaning of the third litter; for other animals, we focused video recording on 3-5 days before birth up until P5.

Behavioral Analysis. For analysis of video data, we used two different DeepLabCut (DLC) networks: one trained to track the location of the dam and another one to track the pups. The location of the nest was extracted with custom Matlab code using image segmentation, and we processed frame-by-frame pose data obtained with DLC to identify episodes of dam nest entry or exit. Nest entry time was defined as the moment when the two front paws entered the nest cavity. We also used nests as a region of interest (ROI), to identify when a pup was out of the nest and how long it took for the dam to it.

We used Datavyu to manually score other behaviors. Five independent coders identified relevant individual and social behaviors and scored the videos blind to litter number and survival rate. The results from each rater were compared, compiled, and cross-validated.

Thermal Imaging. For thermal imaging, we recorded continuous thermograms with a thermal camera (FLIR C3) placed above the nest, streaming temperature data (15 frames per second) to a

Windows PC through a USB cable. Thermographic data were captured with the FLIR Tools software, timestamped, and saved as a csv file.

We synchronized the thermograms and the video recordings post-hoc using timestamp information. We isolated the episodes in which the dam was out of the nest, identified the video frame and thermogram corresponding to the onset of the episode, located the nest opening using image segmentation, and then extracted and averaged the corresponding pixel temperature information from that ROI. We saved these frame-by-frame average traces as csv files. For pup temperature analysis, we identified episodes in which pups were out of the nest and followed a similar procedure to the nest temperature analysis focusing on the out-of-nest pup as our ROI.

Litter Mortality. We monitored both the videos and the thermograms daily to establish how many pups were born alive, how many were stillbirths, and how many were cannibalized. We used color, temperature, movement, and physical integrity to determine if pups were alive. We calculated the litter survival rate as surviving pups on P5 divided by the total number of pups born alive.

Co-Housing. Dams with 0% litter survival during their first litter and less than 50% survival during their second litter were co-housed either with an experienced high-pup-survival dam with her pups (~P5 and older), a virgin, a paternal male, or left in isolation. Co-housing was performed for 16-20 days, before being mated a third and fourth time.

Data and Code Availability. The data that support the findings of this study are available on Databrary. Custom code are available on Github.

Figures and Figure Legends

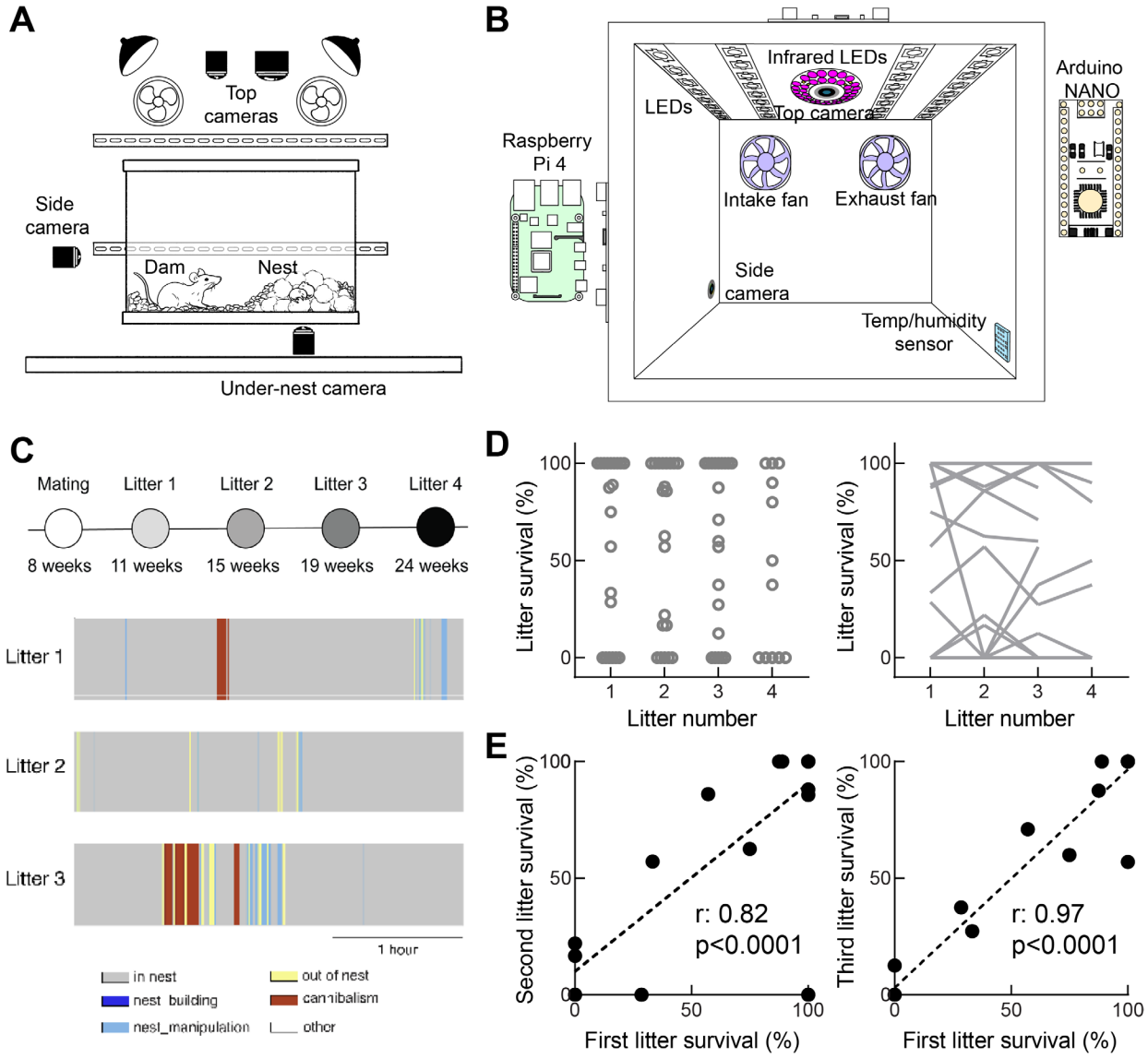

Figure 1. Continuous long-term monitoring of mouse maternal care across litters. **A**. Schematic of setup for 24/7 continuous monitoring of homecage behaviors. A standard mouse homecage was placed within a custom enclosure containing cameras from top, side, and underneath the nest. **B**. System for long-term continuous visible-light and thermal imaging with environmental controls. **C**. Homecage behaviors were monitored over four litters, scored by automated methods (in/out of nest) and manually (nest building, nest manipulation, cannibalism). Top, timeline of monitoring

of individual animals (times are ages of the dams). Bottom, example ethograms showing several hours of spontaneous homecage behaviors for the same animal during litters 1, 2, and 3. **D**. Litter survival at 24 hours post-parturition. Left, fraction of pups surviving after the first postnatal day (*N*=21 dams). Each gray circle represents one dam's litter, with the same animals monitored over litters 1-4. 9/21 dams had 100% survival at litter 1 ('high-pup-survival dams'), and 8/9 of those animals had 100% survival at litter 3 as well. 6/21 dams had 0% litter survival at litter 1, and 5/6 of those animals had 0% survival at litter 3. Right, same data with lines connecting litter survival from the same dam across all 3-4 monitored litters. **E**. Correlation of litter survival over litters. Left, litter 1 survival predicted litter 2 survival across dams (N=21, r(DF): 0.82, $p<0.0001$). Right, litter 1 survival also predicted litter 3 survival (N=21, r(DF): 0.97, $p<0.0001$).

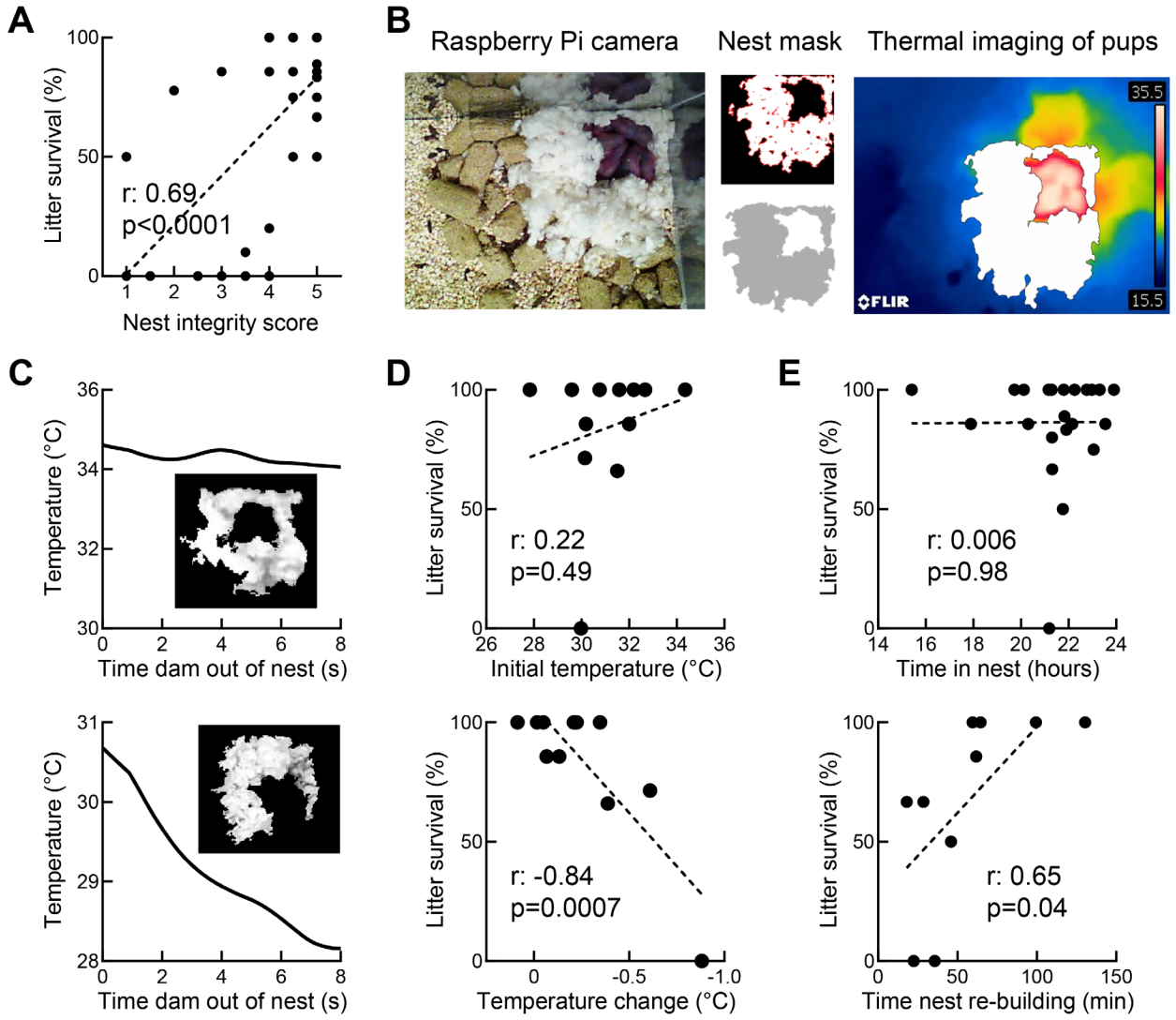

Figure 2. Nests must be rebuilt to ensure pup thermoregulation. **A**. Visual scoring of nest quality; higher nest integrity scores were correlated with higher litter survival rates (r(DF)=.69, p<.001). **B**. Procedure for monitoring pup temperature. Video frames were used to compute nest mask, registered with thermal imaging to isolate pup temperatures. **C**. Examples of heat loss when dam leaves the nest; curves show average drop in pup temperature over many excursions of the dam from the nest (8+ seconds in duration). Top, high-pup-survival dam; bottom, low-pup-survival dam. Insets show representative nest structures from these animals. **D**. Pup temperature when dam left the nest. Top: litter survival was not correlated with the initial temperature upon dam leaving,

*r(*DF)=.22, *p*=0.49. Bottom: litter survival was correlated with temperature drop after dam left the nest, *r*(DF)=-.84, *p*=.007). **E**. Dam interaction with the nest. Top: litter survival was not correlated with total time spent in the nest on the first full day after parturition, *r*(DF)=.006, *p*=.98. Bottom: litter survival was correlated with amount of time dams spent rebuilding the nest, *r*(DF)=.65, *p*=.04.

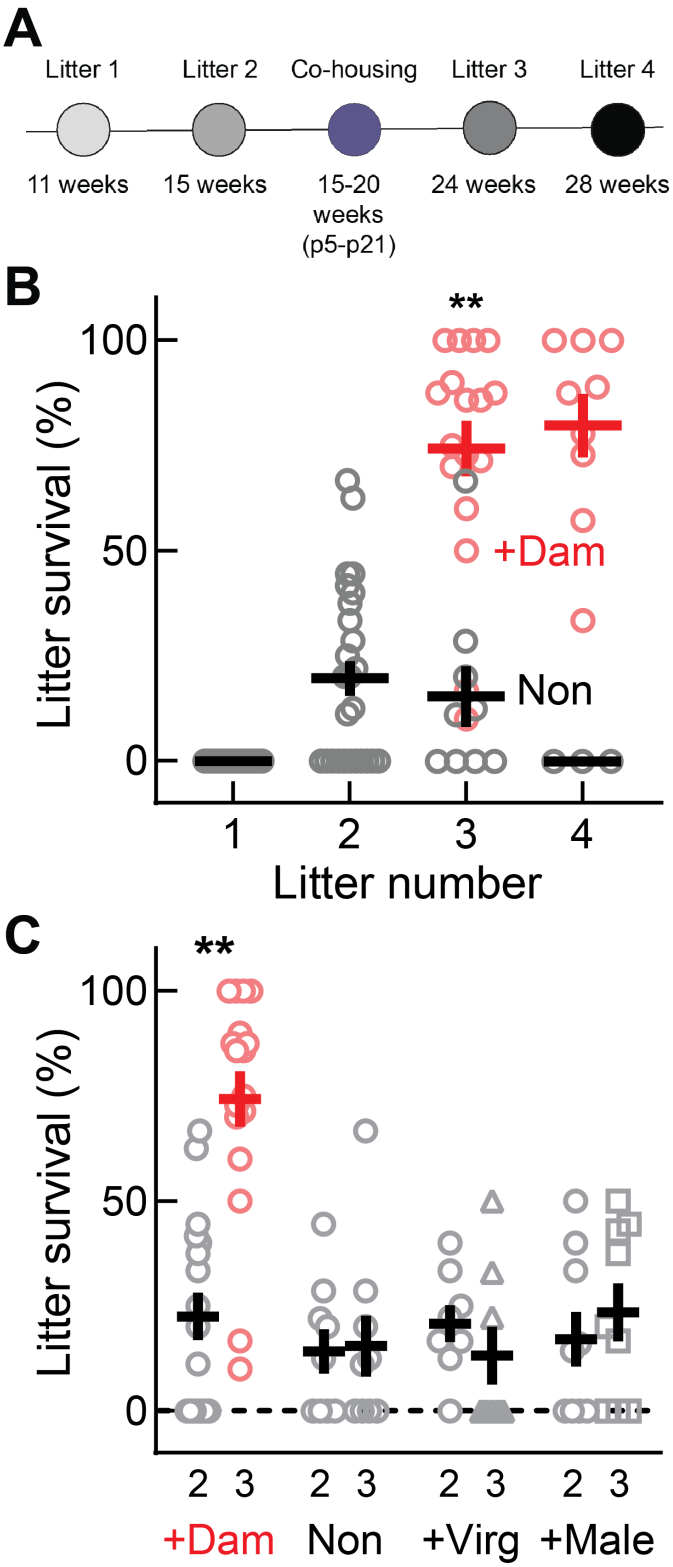

Figure 3. Co-housing with an experienced high-pup-survival dam improves maternal care. **A**, Timeline for co-housing studies: virgin females were bred and those dams with 0% litter survival

411 for litter 1 and <50% survival for litter 2 were selected for these studies; dams were singly-housed
412 for all four litters. Between litters 2 and 3, dams were divided into four groups and placed in a
413 separate cage for 16-20 days before the third round of mating ('+Dam': housed with an
414 experienced high-pup-survival dam and her litter, N=17; 'Non': non-co-housed, i.e., singly housed
415 without pups, N=9; '+Virg': co-housed with a pup-naïve virgin female but no pups, N=8; '+Male':
416 co-housed with an experienced paternal male but no pups, N=9). **B**, Co-housing with an
417 experienced high-pup-survival dam led to improved litter survival of initially low-pup-survival
418 dams. Litter survival of all animals for litter 1: 0%, for litter 2: 19.6±4.1% (N=26, combining the
419 non-co-housed animals with those that were then co-housed with an experienced dam). Seventeen
420 of these animals were co-housed prior to mating for litter 3; litter survival for litter 3: 74.2±6.6%
421 (N=17), for litter 4: 79.7±7.5% (N=9). For the nine non-co-housed animals from the same cohort,
422 litter survival remained low; for litter 3: 15.4±7.3% (N=9, $p<10^{-5}$ compared to litter 3 survival of
423 co-housed animals, Student's unpaired two-tailed t-test), for litter 4: 0% (N=3). Litter 2 survival
424 for these two groups was not significantly different (dams to be co-housed, litter 2 survival:
425 22.5±5.7%; non-co-housed dams, litter 2 survival: 14.2±5.3%; p=0.30, Student's unpaired two-
426 tailed t-test). **C**, Comparison of litter survival for litters 2 and 3 per group ('+Dam', litter 2 survival:
427 22.5±5.7%, litter 3 survival: 74.2±6.6%, N=17, $p<10^{-4}$, Student's paired two-tailed t-test; 'Non',
428 litter 2 survival: 14.2±5.3%, litter 3 survival: 15.4±7.3%, N=9, p=0.86; '+Virg', litter 2 survival:
429 20.8±4.4%, litter 3 survival: 13.2±7.0%, N=8, p=0.29; '+Male', litter 2 survival: 17.1±6.5%, litter
430 3 survival: 23.5±6.9%, N=9, p=0.32). **, p<0.01; *, p<0.05.

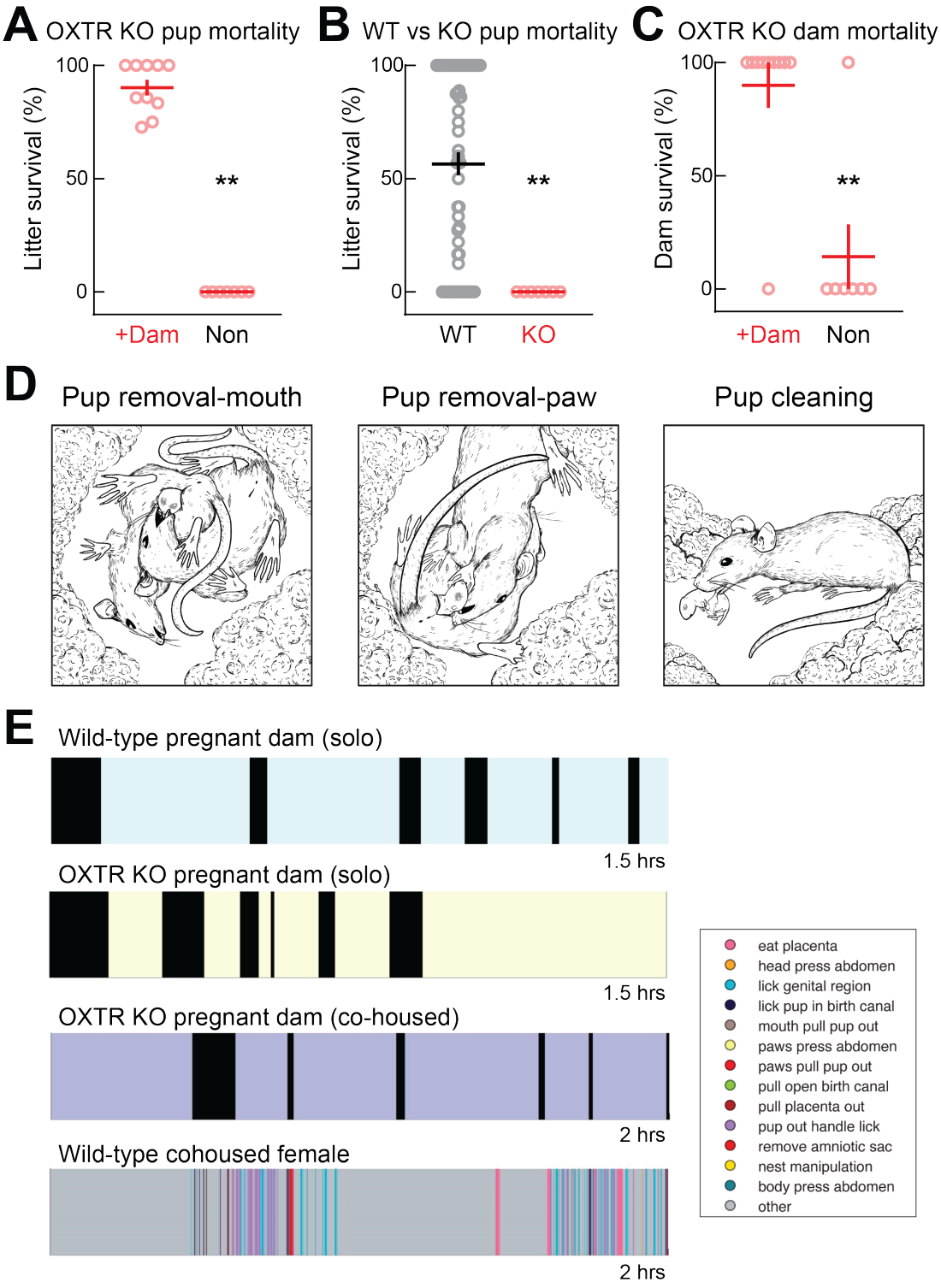

Figure 4. Pregnant wild-type females perform behaviors to help improve maternal-infant survival of oxytocin receptor knockout mice in labor. **A**, Litter survival rates of pups from primiparous oxytocin receptor knockout dams, either co-housed with a pregnant wild-type female (red,

435 ‘+Dam’; 90.2±3.5% litter survival, N=10) or singly-housed (black, ‘Non’; 0% survival, N=7,
436 $p<10^{-5}$, Student’s unpaired two-tailed t-test). **B**, Overall litter survival rates of singly-housed wild-
437 type dams (‘WT’, 56.5±5.1% pup survival across all litters examined here of low- and high-pup-
438 survival dams, N=73) vs oxytocin receptor knockouts (0%, N=7, $p<10^{-5}$). **C**, Maternal survival of
439 oxytocin receptor knockout animals co-housed with a wild-type pregnant female (red, ‘+Dam’,
440 9/10 knockout dams survived labor) or non-co-housed (black, ‘Non’, 1/7 knockout dams survived
441 labor, $p<10^{-5}$, Fisher’s two-tailed exact test). **D**, Illustrations of three main helping behaviors
442 during labor: removing stuck pups with mouth, removing stuck pups with paw, cleaning pups post-
443 delivery. **E**, Ethograms for three different animals in parturition: a singly-housed wild-type dam
444 (top), a singly-housed oxytocin receptor knockout dam (upper middle), and a co-housed oxytocin
445 receptor knockout dam (lower middle) together with a co-housed wild-type female (bottom). For
446 dams, black bars indicate duration of parturition for each birthed pup over 1.5-2 hrs. For co-housed
447 female, colored lines indicate different ‘midwife’-like behaviors denoted by the legend.